SHORT COMMUNICATION

Control of an Organophosphate-Resistant Strain of *Boophilus microplus* (Acari: Ixodidae) Infested on Cattle After a Series of Dips in Coumaphos Applied at Different Treatment Intervals

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ABSTRACT Efficacy of multiple dip treatments (one to three) at 0.3% active ingredient (AI) coumpahos applied at 7- or 10-d intervals was determined against organophosphate (OP)-resistant *Boophilus microplus* (Canestrini). None of the treatments totally prevented repletion of all females. In the 7 d after one treatment, the percentage of reduction of female ticks was 24.8%, whereas in the 10 d after one treatment, the reduction of female numbers was 47.1%. Application of two or three dips at either interval provided substantially higher reductions in female numbers than either single treatment (range 80.2–88.8%). Percentage reduction of the index of fecundity of females (designated as percentage of control) in the 7 d after one dip provided 46.8% control, whereas control in the 10 d after one treatment was 65.6%. Again, application of two or three dips at either interval provided substantially higher control (range 92.9 to >99.9%). Although control levels after two or three dips were similar, only application of three dips at either interval provided a high enough level of control (>99.5%) to ensure that the U.S. *Boophilus* eradication program would not be at risk of inadvertently dispersing viable ticks. Thus, although no treatment regime prevented repletion of all treated females, application of three dips at either interval virtually eliminated production of reproductively active females, thereby eliminating the possibility that ticks would become established. It should be noted that ticks possessing different OP resistance mechanisms than ticks in this study could have higher reproductive capabilities than were observed against these treatment regimes.

KEY WORDS *Boophilus microplus*, insecticide resistance, organophosphate, coumaphos, dipping vat

THE INTENSIVE USE OF organophosphate (OP) acaricides in Mexico in the 1970s created OP resistance in many *Boophilus microplus* (Canestrini) populations in numerous states (Aguirre et al. 1986). Because the United States Cattle Fever Tick Eradication Program (CFTEP) still relies almost exclusively on the use of the OP acaricide coumaphos to eliminate cattle fever tick outbreaks (Graham and Hourrigan 1977), the development of widespread OP resistance in Mexican tick populations is a major concern, because the CFTEP seeks to prevent the reestablishment of cattle fever ticks within the U.S. borders. Under the present procedures required by the CFTEP, cattle presented at ports of entry for importation into the United States from Mexico must be inspected and treated in a dip-

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ping vat charged at a concentration of 0.3% active ingredient (AI) coumaphos (U.S. Department of Agriculture, Animal Plant Health Inspection Service, Veterinary Services 1978). If live *Boophilus* ticks are found on any of the animals within a herd, the animals are dipped but not allowed to move into the United States. These cattle may be represented for importation after a period of not <10 d. Cattle that are represented are again inspected and if no live ticks are detected, the cattle are allowed entrance into the United States. However, if live ticks are found at the second presentation, cattle are dipped but not allowed to pass into the country, and they may not be presented for importation again. Recent studies have demonstrated that a single dip vat treatment in coumaphos against a highly OP-resistant strain of *B. microplus* is not sufficient to eradicate all viable ticks from infested cattle, even at a concentration that is twice the level (0.6% [AI] coumaphos) required by the CFTEP (Davey and George 1999, Davey et al. 2003).

The purpose of this study was to determine whether multiple dipping treatments with coumaphos at a concentration of 0.3% (AI) at different treatment intervals would be sufficient to eliminate OP-resistant ticks from infested cattle. Considering that under present

CFTEP requirements there is a strong likelihood that the presence of OP-resistant ticks at U.S. ports of entry could compromise the program by inadvertently allowing undetected tick infested cattle to pass into the United States, the results of this study could be critical in preventing dispersal of ticks into uninfested areas of the country.

Materials and Methods

The OP-resistant strain of *B. microplus* used in this study was collected in 1998 from cattle near Champoton, Campeche, Mexico, and maintained on live calves in the laboratory. At the time the strain was brought from the field and colonized at the USDA–ARS, Cattle Fever Tick Research Laboratory, Mission, TX, the lethal concentration for 50% of treated ticks (LC_{50}) was determined to be 0.656% (AI) coumaphos, which was 12 times greater than the LC_{50} of a susceptible strain. Since the laboratory colonization of the strain, it has been selectively pressured with coumaphos during virtually all subsequent generations (22 generations), but the level of resistance has remained virtually the same (0.688% [AI] coumaphos) as it was at the beginning of laboratory colonization. This strain of ticks was also used by Davey et al. (2003), having a reported level of resistance that was 13 times higher than that of the LC_{50} of a susceptible strain.

Sixteen Hereford heifer calves, each weighing ≈200 kg, were used in the study. Throughout the study (September–October), each calf was held separately in a stanchion in a 3.3 by 3.3-m stall inside an open-sided barn under ambient conditions, although no direct sunlight or rainfall reached the animals. The calves were randomly divided into four groups, each containing four animals. Each calf was infested on three separate occasions with OP-resistant larvae at 20, 13, and 6 d before the initial coumaphos treatment. Each larval infestation consisted of ≈5,000 individuals (derived from a vial containing 250 mg of eggs) that were 3–5 wk old, such that at the time of the initial treatment each of the 16 calves was infested with all parasitic stages (adults, nymphs, and larvae) of the OP-resistant tick strain.

All treated cattle used in the study were immersed in an 11,336-liter capacity concrete dipping vat (total immersion plunge vat) charged with coumaphos at a concentration of 0.3% (AI). Calves in one group were dipped three times at intervals of 7 d between each treatment (total of 14 d to obtain three dips). Calves from a second group were also dipped three times, except that the interval between each treatment was 10 d (total of 20 d to obtain three treatments). The remaining two groups of cattle were not treated and were thus combined into a single group of eight animals that served as an untreated control.

All engorged female ticks that detached from each calf were collected beginning on the day after the initial treatments and continuing for 25 d consecutively thereafter. The 25-d period for collecting ticks after the initial treatment was made based on Hitchcock's (1955) report that >99% of all larval ticks in-

fested at a given time will detach within 31 d after infestation. Thus, because day 25 after the initial treatment was the 31st d after the last infestation was made, it ensured that virtually all ticks on the calves would have detached by this time. On each day of the 25 d after initial treatments, a random sample of up to 10 females per calf per day (whenever possible) was collected to obtain ovipositional data on the ticks. The ticks within each sample (≤10) were washed, dried, and weighed collectively, placed in a petri dish (9 cm in diameter), and maintained in an incubator at 27 ± 2°C, 92% RH, and a photoperiod of 12:12 (L:D) h for 20 d. After oviposition was complete (20 d), the egg mass produced by females in each sample was weighed and placed in a shell vial (25 by 95 mm; 8 dram), returned to the incubator, and allowed 4 wk to complete hatching. After hatching was completed (4 wk), the percentage of egg hatch was estimated by visually comparing the proportion of larvae to the proportion of unhatched eggs within each vial. After all data had been collected over the 25-d evaluation period, the index of fecundity (IF) of the ticks recovered from each calf (untreated and treated groups) on each day was calculated using the formula reported by Davey et al. (2001), which is derived from the index of reproduction (IR), as described by Drummond et al. (1969).

After all data were collected over the 25-d evaluation period, the number of ticks per calf per day obtained from each of the two treated groups was summed over the number of days after each of the three treatments (either 7- or 10-d treatment intervals). These values, obtained from each of the treated groups, were compared with the overall mean number of ticks recovered from the untreated group during the same time interval to obtain a percentage reduction in the number of females per calf for the time interval after each of the three treatments at 7- or 10-d intervals. The daily IF values were analyzed in a similar manner. The daily IF values for each calf in each of the two treated groups (either 7- or 10-d treatment intervals) were summed over the number of days after each of the three treatments. These values were then compared with the overall mean IF value for all cattle in the untreated group having the same 7- or 10-d time interval to establish the percentage of reduction of the IF (hereafter designated as the percentage control of the IF) after each of the three treatments by using a modified formula of Abbott (1925), as reported by Davey et al. (2001). It should be noted that the duration of the evaluation period after the third dip treatment was 11 and 5 d for calves treated at 7-d treatment intervals or calves treated at 10-d treatment intervals, respectively, because virtually all of the engorged female ticks would have detached during these respective time intervals after the final dip treatment (Hitchcock 1955).

The measured variables (percentage reduction in number of ticks per calf and percentage control of the IF) were analyzed by General Linear Model (GLM), one-way analysis of variance by using SAS software (SAS Institute 1987). Differences among means within

Table 1. Mean ± SD number of ticks per calf, IF, percentage of reduction in number of ticks per calf, and percentage control of the IF for female ticks recovered from untreated and treated cattle infested with an organophosphate-resistant strain of *B. microplus* ticks after each of three dips at a concentration of 0.3% active ingredient (AI) when treatments were applied at 7- or 10-d intervals

No. of dips	Evaluation period after each indicated dip (Days)	No. of females per calf for untreated group	No. of females per calf for treated group	IF for untreated group	IF for treated group	Reduction in no. of females per calf (%)	Control of the IF (%)
			7-Day Interval between Each Dip				
1	7	1,092 ± 188	821 ± 74	93.1 ± 24.8	49.5 ± 13.6	24.8 ± 6.8a	46.8 ± 14.7a
2	7	989 ± 264	188 ± 83	70.6 ± 21.4	5.0 ± 1.7	81.0 ± 8.4c	92.9 ± 2.4c
3	11[a]	1,254 ± 323	141 ± 30	90.8 ± 18.2	0.1 ± 0.1	88.8 ± 2.4c	>99.9 ± 0.1c
			10-Day Interval between Each Dip				
1	10	1,737 ± 400	1,013 ± 135	144.0 ± 34.0	49.6 ± 8.1	47.1 ± 7.8b	65.6 ± 5.7b
2	10	1,290 ± 140	256 ± 90	94.9 ± 20.0	4.2 ± 3.2	80.2 ± 7.0c	95.6 ± 3.4c
3	5[b]	308 ± 216	35 ± 9	15.5 ± 11.5	0.1 ± 0.1	88.6 ± 2.8c	99.6 ± 0.7c

Means within each of the last two columns followed by the same letter are not significantly different ($P < 0.05$; Ryan–Einot–Gabriel–Welch multiple range test). Reduction in no. of females per calf (%): $F = 74.6$; df = 5, 18; $P < 0.0001$. Control of the IF (%): $F = 44.2$; df = 5, 18; $P < 0.0001$.

[a] Evaluation period after all engorged females were collected from treated cattle after the third dip at 7-d dipping intervals.
[b] Evaluation period after all engorged females were collected from treated cattle after the third dip at 10-d dipping intervals.

each measured variable for each treatment group were determined by use of the Ryan–Einot–Gabriel–Welsch multiple range test. Values were considered significant at a value of $P < 0.05$.

Results

Analysis of results showed that the percentage reduction in the number of ticks per calf was significantly affected ($F = 74.6$; df = 5, 18; $P < 0.0001$) by the number of dips and treatment interval between each dip (Table 1). In the 7 d after a single dip at 0.3% (AI) coumaphos, the reduction of OP-resistant ticks was only 24.8%, compared with ticks obtained from untreated calves and was significantly lower ($P < 0.05$) than the reduction of tick numbers observed in the 10 d after a single dip (47.1%). Likewise, the reduction in the percentage of surviving females in the 10 d after one dip was significantly lower ($P < 0.05$) than that of any multiple dips at either 7- or 10-d treatment intervals (range 80.2–88.8% reduction), where no differences ($P > 0.05$) were observed.

The percentage of control of the IF of OP-resistant females followed the same trend as that of the percentage reduction in the number of females per calf, producing significant differences ($F = 44.2$; df = 5, 18; $P < 0.0001$) that were dependent on the number and treatment interval of the dips (Table 1). The lowest level of control was achieved in the 7 d after a single-dip treatment at 46.8% control. In the 10 d after a single dip treatment at 0.3% (AI) coumaphos, the level of control (65.6%) was significantly higher ($P < 0.05$) than at 7 d after a single dip, but both single dips at 7- or 10-d treatment intervals provided significantly lower control ($P < 0.05$) than was achieved after two or three dips were applied, regardless of the treatment interval. Two or three dips at both 7- and 10-d treatment intervals provided 92.9–99.9% control of the IF with no differences ($P > 0.05$) among any of the means.

Discussion

Results of this study clearly demonstrate that neither single- nor multiple-dip treatments will be totally effective in preventing the repletion of all female ticks at either treatment interval (7 or 10 d apart). In the 7 or 10 d after a single 0.3% (AI) coumaphos dip, large numbers of OP-resistant *B. microplus* will survive to repletion on treated cattle, and the reproductive capacity (IF) of these females will be only marginally affected. Conversely, two or three coumaphos treatments applied at 7- or 10-d intervals against OP-resistant ticks will have a substantial adverse impact on the number of females that survive, and the reproductive capacity of these females will be dramatically lower than that of untreated female ticks. Even though there was no statistical difference ($P > 0.05$) in the level of control of the IF obtained after two dips at 7- or 10-d intervals, compared with the control obtained after three dips, the three dip treatments at both intervals were the only treatment regimes that controlled the reproductive capacity (IF) of the surviving females by >99%, thereby making it highly unlikely that enough OP-resistant ticks would be produced to establish an infestation that would jeopardize the success of the CFTEP.

Our findings stand in stark contrast to the level of control that is obtained against OP-susceptible *B. microplus* ticks, where even a single dip applied at 0.165% (AI) coumaphos results in virtually no engorged female ticks being able to survive after the 7th d after treatment, and the ability of any females to produce viable eggs is totally prevented (Davey et al. 1997). Our results also contrast with other studies conducted against OP-resistant *B. microplus.* Davey and George (1999) reported that from the 8th through the 21st d after a single dip at 0.279% (AI) coumaphos, numbers and reproductive capacity of OP-resistant females recovered from treated cattle were similar, although somewhat lower, than the results obtained in this study after two or three coumaphos dips. However,

the probable reasons for the similarity in control between this study after two or three dips and results of Davey and George (1999) after only one dip are that they used a lower treatment concentration, and more importantly, the ticks used in their study were ≈27% less resistant to coumaphos than the ticks used in this study. In a more recent study using the same OP-resistant strain of ticks used in this study, Davey et al. (2003) reported that tick numbers and reproductive capacity of females from the 8th to the 21st d after a single dip at 0.299% (AI) coumaphos applied while ticks were either nymphs and larvae were similar to results obtained in this study after two or three dips at 0.3% (AI) coumaphos at comparable time intervals. This seems to suggest that the application of a second or third dip against a highly OP-resistant strain of *B. microplus* will have only a minimal added effect in reducing tick numbers and reproduction, compared with applying only a single treatment. However, the level of control (reduction of the IF) achieved in this study after two or three dips, although similar to Davey et al. (2003) at the same time intervals, was still higher than they obtained, and in addition, the level of control of the IF in their study never reached the 99% level, as it did in this study. Achieving a level of control that is ≥99% is of critical importance when eradication is the goal because any production of reproductively active females poses a distinct risk that viable ticks will be able to establish themselves in areas outside the permanent quarantine zone established by the CFTEP.

From the perspective of the CFTEP, the results of this study provide a positive, although indirect, indication that eradication of OP-resistant *B. microplus* would be possible by using coumaphos under the proper circumstances. The ability of some engorged females to survive to repletion, even after being subjected to three coumaphos dips, would still be a factor of grave concern to the eradication program because the decision to allow cattle to pass into the United States is based on the total absence of any live ticks on the animals. Thus, the presence of live females on the animals treated three times, regardless of whether these females were capable of producing viable offspring, would result in the rejection of movement of the animals into the country. However, based on the results of the study the application of three dips at 7- or 10-d intervals at 0.3% (AI) would provide >99.5% control of the reproduction of any ticks that did survive the three dips. Thus, in the event cattle that were presented at any U.S. port of entry that were actually infested with OP-resistant ticks, but the ticks were not detected during the inspection process, then if these cattle were subjected to a series of three dips at 7- or 10-d intervals, there would still be a very high likelihood that the treated females would produce no viable offspring. Obviously, holding cattle at port facilities for a 14–20-d period to allow multiple treatments to be accomplished would create a financial and logistical hardship on the eradication program. However, considering the fact that coumaphos is the only approved acaricide, and there are no alternative chemicals presently available, the retention and multiple treatment of cattle at the port may be the only means of ensuring the integrity of the eradication program and eliminating the risk of inadvertently dispersing viable OP-resistant ticks to uninfested areas of the United States.

It seems worthy of note to also mention that although the ticks evaluated in this study failed to produce viable offspring after being subjected to three dip treatments, it is possible that a tick strain possessing a different OP resistance mechanism from the strain tested could have higher reproductive capabilities against the treatment regimes tested than the strain of ticks used in this study. Therefore, vigilance is still of great importance in the cattle inspection process to prevent the inadvertent movement of OP-resistant ticks into the country. Because different OP resistance mechanisms are identified in *B. microplus* ticks, additional research will be critical in evaluating the efficacy of different treatment scenarios against ticks with different resistance mechanisms to ensure the continued success of the eradication program.

Acknowledgments

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